
Research Article

Topological Modeling of 1H-indole-2,3-dione Analogues as *in vitro* anti-cancer agents

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Abstract: QSAR analysis on a set of synthesized 1H-indole-2, 3-dione Analogues tested for growth inhibitory anticancer activity was performed by using MLR procedure. The activity contributions of these compounds were determined from regression equation and the validation procedures to analyze the predictive ability of QSAR models were described. The results are discussed on the basis of statistical data. High agreement between experimental and predicted anticancer activity inhibitory values is obtained. The results of this study indicate that the substitution of electron withdrawing group, aromatic ring, polarizability, etc. parameters has a significant effect on anticancer activity of this class of compounds, thus simplifying design of new biological active molecule.

Keywords: QSAR, anticancer activity, *in vitro* studies.

INTRODUCTION

The 1H-indole-2, 3-dione analogues moiety are a large chemical which is responsible for a broad spectrum of biological properties in many synthetically versatile molecules [1–5]. Among these properties, antifungal, antitubercular, antioxidant, antiallergic, cytotoxic and antineoplastic activities of this moiety have been found to be interesting [6-10].

Pharmacokinetic studies of 1H-indole-2, 3-dione analogues moiety in humans will be undertaken in which the concentration–time profile of the drug in blood will be measured at frequent intervals in order to establish the rate at which the drug is absorbed and eliminated. These studies together, if successful, will permit multiple-dose studies to be undertaken.

There are many software packages that calculate wide sets of different theoretical descriptors. The greatest advantage of theoretical descriptors is the fact that they can be calculated homogeneously by defined software for all chemicals, including those not yet synthesized but represented by a hypothesized chemical structure, and therefore they are reproducible. A variety of methods for building QSAR models exists.

These methods are called pattern recognition methods because their aim is to devise algorithms that could learn to distinguish patterns in a data set. They can be classified as supervised (for example, Multiple Linear Regression, Discriminant Analysis, Partial Least

Squares, Classification and Regression Trees, Neural Networks, etc.) or unsupervised (for example, Principal Component Analysis, Cluster Analysis, k-Nearest Neighbours, Nonlinear Mapping, etc.), where supervision refers to the use of the response data which are being modeled.

The numerical descriptors are responsible for encoding important features of the structure of the molecules and can be categorized as electronic, geometric, hydrophobic, and topological characters. Descriptors were calculated for each compound in the data set using the software ChemSketch and E-Dragon 7.1.

5, 7-dibromoisatin of 1H-indole-2, 3-dione analogues moiety significantly more potent as a cytotoxin than U₉₃₇ (human monocyte-like histiocytic lymphoma) cells [11], its N-benzyl derivatives with more cytotoxicity toward these lymphoma cells and activity against a range of human cancer cell lines including a metastatic breast adenocarcinoma cell line (MDA-MB-231) [12], and cytotoxic N-alkylhaloisatins are some examples of reported anti-cancer halogenated isatins in recent researches [13–16]. The complete regression analysis were carried out by PASS 2005, GESS 2006, NCSS Statistical softwares.

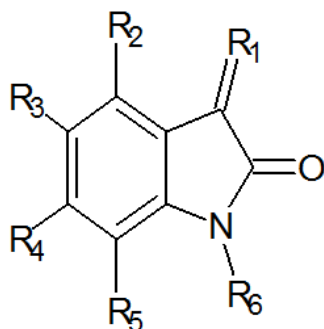


Fig-1:Chemical structure of 1H-indole-2, 3-dione analogues moiety derivatives for anticancer activity

An important approach of the researchers in modification of the 1H-indole-2, 3-dione analogues moiety has been to establish a comprehensive structure–activity relationship (SAR), for this class of anti-cancer agents. It has been shown that the introduction of electron-withdrawing halogens to the benzene ring of the 1H-indole-2, 3-dione analogues moiety molecule is associated with increased biological activity [17].

Introduction of an aromatic ring with one or three carbon atom linker at N enhances the activity too [18]. The structure activity relationship of anti-cancer halogenated isatins is defined in some extent in the literature, but there is not any report for a quantitative approach, quantitative structure activity relationship (QSAR), to illustrate this relationship more precisely and in details. In this context, it was of interest to us to investigate the QSAR of 1H-indole-2, 3-dione analogues moiety derivatives that have been reported to exhibit anti-cancer activity in recent reports. Our QSAR analysis establishes mathematical relationship between biological activities and computable parameters such as topological, physicochemical, stereochemical or electronic indices.

COMPUTATIONAL METHODS

Data Set

The biological data used in this study were anti-cancer activity against U_{937} , (in terms of $-\log IC_{50}$), of a set of forty seven 1H-indole-2, 3-dione analogues moiety derivatives [19–22] are taken from the research article published by Afshin Fassihi *et al.* on European journal of medicinal chemistry 45 (2010) 1113-1118. The structural features activities of these compounds are listed in Table 1. Calculated descriptors for each molecule are summarized in Table 2.

Molecular Descriptors

Various 2D descriptors like element counts, topological index, Baumann alignment independent topological descriptors *etc.*, were calculated using Dragon software. The preprocessing of the independent variables (i.e., descriptors) was done by removing invariable (constant column) and cross-correlated

descriptors which resulted in total descriptors for QSAR respectively to be used for QSAR analysis.

Selection of Training and Test Set

The dataset of 47 molecules was divided into training and test set by Sphere Exclusion (SE) method for QSAR models with pIC_{50} activity field as dependent variable and various 2D descriptors calculated for the molecules as independent variables.

This is done to test the internal stability and predictive ability of the QSAR models. Developed QSAR models were validated by the following procedure:

Internal Validation

Internal validation was carried out using leave-one-out (R^2 , LOO) method. For calculating R^2 , each molecule in the training set was eliminated once and the activity of the eliminated molecule was predicted by using the model developed by the remaining molecules.

The R^2 was calculated using the equation which describes the internal stability of a model. where \hat{y}_i and y_i are the actual and predicted activity of the i^{th} molecule in the training set, respectively, and \bar{y} mean is the average activity of all molecules in the training set.

External Validation

For external validation, the activity of each molecule in the test set was predicted using the model developed by the training set. The R^2 value is calculated as follows. Where, \hat{y}_i and y_i are the actual and predicted activity of the i^{th} molecule in the training set, respectively, and \bar{y} mean is the average activity of all molecules in the training set.

Both summations are over all molecules in the test set. Thus, the R^2 value is indicative of the predictive power of the current model for external test set.

Randomization Test

To evaluate the statistical significance of the QSAR model for an actual dataset, one tail hypothesis testing was used [23-26]. The robustness of the models for training sets was examined by comparing these models to those derived for random datasets.

Random sets were generated by rearranging the activities of the molecules in the training set. The statistical model was derived using various randomly rearranged activities (random sets) with the selected descriptors and the corresponding R^2 were calculated. The significance of the models hence obtained was derived based on a calculated Z score.

Where, h is the R^2 value calculated for the actual dataset, μ the average R^2 , and σ are its standard deviation calculated for various iterations using models build by different random datasets. The randomization

test suggests that all the developed models have a probability of less than 1% that the model is generated by chance.

QSAR

Multiple regressions are the standard method for multivariate data analysis. It is also called as ordinary least squares regression (OLS). This method of regression estimates the values of the regression coefficients by applying least squares curve fitting method. For getting reliable results, dataset having typically 5 times as many data points (molecules) as independent variables (descriptors) is required. The regression equation takes the form

$$Y = b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + c$$

Where, Y is the dependent variable, the 'b's are regression coefficients for corresponding 'x's (independent variable), 'c' is a regression constant or intercept.

In the present study QSAR model was developed using multiple regression by forward-backward variable selection method with pIC₅₀ activity field as dependent variable and topological and physicochemical descriptors as independent variable having cross-correlation limit of 1. Selection of test and training set was done by sphere exclusion method.

Table.1 Structural Substitutions of 1H-indole-2, 3-dione analogues moiety derivatives for anticancer activity

S.No.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
1	O	H	Br	H	Br	H ₂ CCH=CH ₂
2	O	H	Br	H	Br	H ₂ CCH ₂ OCH ₃
3	O	H	Br	H	Br	H ₂ CCH ₂ CH(CH ₃) ₂
4	O	H	Br	H	Br	H ₂ CC ₆ H ₅
5 ^b	O	H	Br	H	Br	H ₂ CC ₆ H ₄ CH ₃ ^c
6	O	H	Br	H	Br	H ₂ CC ₆ H ₄ OCH ₃ ^c
7	O	H	Br	H	Br	H ₂ CC ₆ H ₄ OCH ₃ ^d
8	O	H	Br	H	Br	H ₂ CC ₆ H ₄ NO ₂ ^c
9	O	H	Br	H	Br	H ₂ CC ₆ H ₄ NO ₂ ^e
10	O	H	Br	H	Br	H ₂ CC ₆ H ₄ Cl ^c
11	O	H	Br	H	Br	H ₂ CC ₆ H ₄ Br ^c
12	O	H	Br	H	Br	H ₂ CC ₆ H ₄ I ^c
13	O	H	Br	H	Br	H ₂ CC ₆ H ₄ CF ₃ ^c
14 ^b	O	H	H	Br	H	H ₂ CC ₆ H ₄ CF ₃ ^c
15	O	H	Br	H	Br	H ₂ CC ₆ H ₄ COOCH ₃ ^c
16	O	H	Br	H	Br	H ₂ CC ₆ H ₄ C(CH ₃) ₃ ^c
17	O	H	Br	H	Br	H ₂ CCH=CHC ₆ H ₅
18	O	H	Br	H	Br	H ₂ CC ₆ H ₄ C ₆ H ₅ ^c
19	O	H	H	H	H	H
20	O	Br	H	H	H	H
21	O	H	Br	H	H	H
22	O	H	H	Br	H	H
23	O	H	H	H	Br	H
24	O	H	F	H	H	H
25 ^b	O	H	I	H	H	H
26	O	H	NO ₂	H	H	H
27	O	H	OCH ₃	H	H	H
28	O	H	Br	H	Br	H
29 ^b	O	H	Br	Br	H	H
30	O	H	I	H	I	H
31	O	H	Br	H	NO ₂	H
32	O	H	Br	Br	Br	H
33	N-C ₆ H ₅	H	H	H	H	H
34	N-C ₆ H ₅	H	Br	H	Br	H
35	O	H	H	H	H	CH ₃
36	O	H	Br	H	Br	H ₂ CCH ₂ C ₆ H ₅
37	O	H	Br	H	Br	H ₂ CCH ₂ C ₆ H ₄ Br ^d
38	O	H	Br	H	Br	H ₂ CCH ₂ C ₆ H ₄ Br ^c
39	O	H	Br	H	Br	H ₂ CCH ₂ C ₆ H ₄ OCH ₃ ^d
40	O	H	Br	H	Br	H ₂ CCH ₂ C ₆ H ₄ OCH ₃ ^c
41	O	H	Br	H	Br	CH ₂ C ₁₀ H ₇ ^f
42	O	H	Br	H	Br	CH ₂ C ₁₀ H ₇ ^g
43	O	H	Br	H	Br	CH ₂ COC ₆ H ₅
44	O	H	Br	H	Br	CH ₂ COC ₆ H ₄ Br ^d
45	O	H	Br	H	Br	CH ₂ COC ₆ H ₄ Br ^c
46	O	H	Br	H	Br	CH ₂ COC ₆ H ₄ OCH ₃ ^d
47	O	H	Br	H	Br	CH ₂ COC ₆ H ₄ OCH ₃ ^c

Table 2: Calculated descriptors of isatin derivatives with experimental anticancer activities pIC₅₀

C.No	pIC ₅₀	MAXDN	TWC	TPC	X ²	X ³	XMOD	X ³ v
1	5.18	2.173	12.282	6.004	6.966	5.897	63.782	3.356
2	5.46	2.171	12.3	6.114	7.319	6.166	67.989	3.563
3	5.65	2.093	12.334	6.215	8.148	6.352	68.917	3.974
4	5.94	2.15	12.467	6.726	8.917	7.605	75.888	4.228
5	6.31	2.149	12.513	6.839	9.539	8.015	78.251	4.506
6	5.74	2.176	12.537	6.942	9.708	8.424	82.594	4.544
7	5.75	2.184	12.545	6.942	9.72	8.349	82.594	4.519
8	6.05	2.69	12.581	7.036	10.438	8.714	85.614	3.974
9	5.64	2.705	12.626	7.036	10.365	8.674	85.715	4.03
10	6.01	2.182	12.513	6.839	9.539	8.015	81.426	4.55
11	6.2	2.16	12.513	6.839	9.539	8.015	86.622	4.827
12	5.64	2.151	12.513	6.839	9.539	8.015	91.818	5.018
13	6.1	5.667	12.661	7.123	11.497	8.952	87.769	4.666
14	5.28	5.657	12.555	7.021	10.947	8.624	76.933	4.05
15	5.92	2.235	12.604	7.123	10.579	9.22	88.636	4.76
16	5.95	2.149	12.661	7.123	11.497	8.952	85.519	5.205
17	5.63	2.161	12.447	6.853	9.612	8.149	81.888	4.442
18	6.12	2.161	12.693	7.58	11.592	10.088	93.686	5.446
19	3.25	2.203	11.64	5.263	4.78	4.215	33.289	1.626
20	3.67	2.225	11.843	5.438	5.318	4.615	44.125	2.356
21	4.19	2.219	11.781	5.438	5.414	4.558	44.024	2.174
22	4.13	2.219	11.779	5.438	5.414	4.551	44.024	2.174
23	4.08	2.225	11.832	5.438	5.308	4.695	44.125	2.364
24	4.01	2.365	11.781	5.438	5.414	4.558	36.518	1.681
25	4.27	2.202	11.781	5.438	5.414	4.558	49.22	2.352
26	3.88	2.615	11.943	5.727	6.313	5.279	43.015	1.913
27	3.38	2.247	11.844	5.591	5.583	4.982	39.996	1.926
28	4.98	2.242	11.966	5.598	5.954	4.946	54.859	2.828
29	4.94	2.236	11.935	5.598	5.922	5.143	54.859	3.604
30	5.11	2.199	11.966	5.598	5.954	4.946	65.252	3.218
31	3.59	2.65	12.144	5.866	6.884	5.522	53.851	2.426
32	5.17	2.259	12.129	5.746	6.331	5.813	65.796	5.158
33	4.12	1.81	12.07	6.384	7.325	6.253	51.454	2.643
34	4.86	1.849	12.301	6.629	8.498	6.985	73.024	3.844
35	3.62	2.115	11.89	5.434	5.19	4.884	36.068	2.009
36	6.11	2.125	12.448	6.791	9.259	7.908	78.888	4.511
37	6.11	2.135	12.498	6.9	9.892	8.228	89.622	5.051
38	6.06	2.133	12.493	6.9	9.88	8.318	89.622	5.11
39	5.97	2.151	12.524	6.999	10.061	8.652	85.594	4.803
40	5.63	2.146	12.518	6.999	10.05	8.726	85.594	4.828
41	6.72	2.156	12.736	7.541	10.812	9.573	87.787	5.253
42	6.13	2.156	12.698	7.541	10.897	9.528	87.686	5.199
43	5	2.349	12.537	6.853	9.788	8.373	81.929	4.442
44	5.2	2.359	12.586	6.957	10.422	8.7	92.664	4.986
45	5.04	2.357	12.581	6.957	10.41	8.784	92.664	5.041
46	5.33	2.375	12.611	7.053	10.591	9.124	88.636	4.738
47	5.27	2.37	12.604	7.053	10.579	9.192	88.636	4.758

RESULTS AND DISCUSSION

In the first step, separate stepwise selection-based QSAR analyses were performed using different types of descriptors, and then, an MLR equation was

obtained utilizing the pool of all calculated descriptors. The resulted QSAR models from different types of descriptors for the compounds (41 molecules as calibration) are listed in Table 3.

After 2D QSAR study by Multiple Linear Regression method using forward-backward stepwise variable selection method, the final QSAR equation

developed QSAR/QSPR models was as follows. The highest correlation coefficient ($r \geq 0.8$) between the descriptors as illustrated in Table 3.

Table 3: Correlation matrix between different descriptors and anticancer activity

	pIC ₅₀	TWC	TPC	X ²	X ³	X ³ v
pIC ₅₀	1.0000					
TWC	0.8618	1.0000				
TPC	0.8310	0.973	1.0000			
X ²	0.8198	0.9774	0.9844	1.0000		
X ³	0.8184	0.9757	0.991	0.9891	1.0000	
X ³ v	0.8876	0.9129	0.8820	0.8829	0.8964	1.0000

QSAR Model No.1

$$pIC_{50} = 2.5394 + 0.6844X^3v$$

The equation 1 shows among topological descriptors the valence connectivity index chi-3 (X³v) have positive effects on cytotoxic activity of the compounds. Among the equations obtained from the whole chemical structures, the equation has the highest statistical parameters. It could explain and predict 88% and 86% of variances in the activity data. The positive coefficient indicating electron with drawing halogen to the benzene ring of the 1H-indole-2, 3-dione analogues moiety derivatives with increased biological activity [27-30].

QSAR Model No.2

$$pIC_{50} = -7.1730 + 0.8573TWC + 0.4669X^3v$$

The second equation 2 was found by using chemical descriptors, which explains the positive effect of polarizability index (steric parameter) and hydration energy of studied compounds on the anti-cancer activity. It can explain and predict more than 80% of variances in the biological activity data from equation qsar model no.2 indicate introduction of an aromatic ring with one or three carbon atom linker at N enhances the biological activity too[31].

The equation 3 demonstrated the effect of constitutional descriptors on the anti-cancer activity of these compounds. Stepwise selection and elimination of variables produced a four-parametric QSAR equation after the deletion of compound no. 8, 12, 15, 31, 33, 34 and 43.

Table 4: Statistical validated and statistical cross validated parameters for testing prediction ability of the MLR models of 1H-indole-2, 3-dione analogues moiety.

Model	n	Intercept	R ²	F-Ratio	SSY	R ² _{CV}	R ² _{ADJ}
1	47	2.5395	0.7879	167.180	30.0740	0.7697	0.7832
2	47	-7.1730	0.8038	30.132	30.6803	0.7729	0.7949
3	47	-4.2956	0.8261	68.092	31.5316	0.7747	0.8140
4	47	-21.1922	0.8390	54.722	32.0242	0.7829	0.8237

The number of secondary amides (aliphatic) (nCONHR) has a negative effect on the anticancer activity. It should be noted that this structure can be found as a cyclic moiety in compounds 19-34 which are isatin derivatives without substitution on N.

QSAR Model No.3

$$pIC_{50} = -4.2955 + 1.8336TPC - 0.6744X^3 + 0.6408X^3v$$

The positive coefficient of QSAR model no.3 indicate that the effect of polarizability index and hydration energy while the negative coefficient indicate and comparing the compounds 36-40 with compounds 43-47 in table 1 with the same substituent in the other part of the molecules but with different no. of secondary C (sp³) at N of the 1H-indole-2, 3-dione analogues moiety ring. This finding will be confirmed very well.

QSAR Model No.4

$$pIC_{50} = -21.1921 + 1.6434TPC + 1.4840TWC - 0.7732X^3 + 0.5449X^3v$$

This equation, which has a high statistical quality demonstrates the positive effect of TWC, TPC, X³v on the biological activity. It shows that increasing the number of halogen atoms of compounds results in an activity enhancement. The result is not helpful because one parameter changing between these pairs of molecules. Substitutions of this nitrogen would enhance the biological activity. The number of ketones (nCO) has negative effects on the anticancer activity of 1H-indole-2, 3-dione analogues moiety. This is in agreement with previous SAR studies by Vine et al. [10]. They believed that introduction of halogen atoms as R₃, R₄ and R₅ enhances the activity with some di- and tri- halogenated 1H-indole-2, 3-dione analogues moiety [32-35].

Comparison of molecules in our data set (e.g. 28 with 43 or 14 with 22) to confirm this result is not helpful because there are more than one parameter changing between these pairs of molecules. Hence, according to E6, substitution of this nitrogen would

enhance the biological activity. The same result was

obtained by Matesic *et al.* [12].

Table-5: Results of Regression Analysis

No.	Parameters Used	T-Value	Ai (1,...,3)	Intercept	SD	R ²
1	X _v ³	0.9749	0.6845	2.5395	0.2148	0.7879
2	TWC X _v ³	0.9658	0.8573 0.4670	-7.1730	0.3282	0.8038
3	TPC X _v ³ X _v ³	0.9627	1.8337 -0.6745 0.6409	-4.2956	0.7092	0.8261
4	TPC TWC X _v ³ X _v ³	0.9589	1.6435 1.4840 -0.7732 0.5450	-21.1922	0.7092	0.8390

All described parameters have positive effects on the anti-cancer activity. The predicted values of the activity for calibration set (by cross-validation) and prediction set are listed and are plotted against the corresponding experimental values in Table 6.

Whilst the data show acceptable prediction, we see that the predicted values of some molecules are the same. The statistical parameters of prediction, listed in Table 4, indicate the suitability of the proposed QSAR model based analysis of molecular descriptors [36-42].

Table-6: Actual and Predicted anticancer activity of 1H-indole-2, 3-dione analogues moiety

Com. No.	Act.pIC ₅₀	Pred.pIC ₅₀	Residual
1	5.18	5.172	0.008
2	5.46	5.27	0.19
3	5.65	5.556	0.094
4	5.94	5.702	0.238
5	6.31	5.78	0.53
6	5.74	5.677	0.063
7	5.75	5.734	0.016
9	6.05	5.354	0.696
10	5.64	5.489	0.151
11	6.01	5.804	0.206
13	6.2	5.955	0.245
14	5.64	6.059	-0.419
16	6.1	5.807	0.293
17	5.28	5.4	-0.12
18	5.92	5.558	0.362
19	5.95	6.101	-0.151
20	5.63	5.554	0.076
21	6.12	6.085	0.035
22	3.25	3.375	-0.125
23	3.67	4.057	-0.387
24	4.19	3.9	0.29
25	4.13	3.902	0.228
26	4.08	3.982	0.098
27	4.01	3.631	0.379
28	4.27	3.997	0.273
29	3.88	3.895	-0.015
30	3.38	3.768	-0.388
32	4.98	4.498	0.482
35	4.94	4.718	0.222
36	5.11	4.711	0.399
37	3.59	4.524	-0.934
38	5.17	5.585	-0.415
39	4.12	4.724	-0.604
40	4.86	5.556	-0.696
41	3.62	3.731	-0.111
42	6.11	5.687	0.423
44	6.11	5.978	0.132
45	6.06	5.933	0.127
46	5.97	5.705	0.265
47	5.63	5.651	-0.021

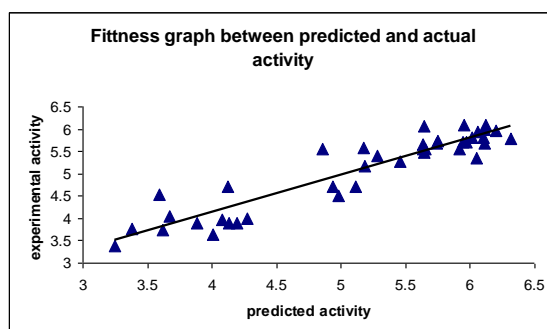


Fig 2: Plots of the validated and cross validated activity against the predicted pIC_{50} and actual pIC_{50} obtained by QSAR Analysis of 1H-indole-2, 3-dione analogues moiety.

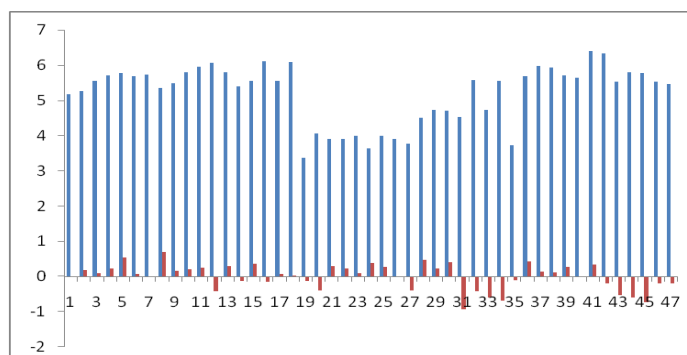


Fig-2: Graph plotted between observed pIC_{50} and residuals of 1H-indole-2, 3-dione analogues moiety ring

CONCLUSIONS

Quantitative relationships between molecular structure and anti-cancer activity of isatin derivatives were discovered by two chemometrics methods: MLR. Different QSAR models revealed that topological parameters have significant impacts on the anti-cancer activity of the compounds.

The Hansch analysis revealed the importance of lipophilic factors as R_3 and R_5 substituents on the anti-cancer activity. A comparison between the two statistical methods employed indicated that MLR represented superior results.

1. Introduction of electron withdrawing halogen to the benzene ring of the 1H-indole-2, 3-dione analogues moiety ring enhance biological anticancer activity.
2. Introduction of an aromatic ring with one or three C atom linker at N enhances biological anticancer activity.
3. The effect of polarizability index and hydration energy enhances anticancer activity.
4. The 1H-indole-2, 3-dione analogues moiety biological anticancer activity shows positivity by the substitution of halogen atoms on R_3 , R_4 and R_7 with some di and tri halogenated derivatives.
5. The positive effect of lipophilic substitution at R_3 and R_5 are favorable for binding affinity.
6. Comparing Compounds 36-40 with 43-47 but N of the 1H-indole-2, 3-dione analogues moiety and the no. of secondary very well.

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